REMARKS

At the outset, applicants would like to thank Examiner Ewoldt for his time and consideration of the present application at the interview of March 6, 2005 with the undersigned attorney. At the interview, the issues raised in the outstanding Official Action were discussed.

In the outstanding Official Action, claims 64-70 were rejected under 35 USC §112, first paragraph, for allegedly not satisfying the enablement requirement.

In imposing the rejection, the Official Action alleged that the specification discloses that only cultures employing 1,000 IU/ml IFN resulted in functional DCs. However, the specification does not disclose that "only" cultures employing the above-identified amount of IFN result in functional DCs. Indeed, the specification teaches that a concentration range of IFN can be used. For example, the Examiner's attention is respectfully directed to page 17, line 30, wherein it is stated that relevant concentrations shall be greater than 100 IU/ml, even if ranges of 500-2,000 IU/ml, 500-1,000 IU/ml and particularly a concentration of 1,000 IU/ml are the most preferred.

Moreover, Figure 3 shows a diagram comparing the effects of different doses of type I IFN. As a result,

applicants believe that the present disclosure is enabling for a concentration range of IFN practice the claimed method.

The Official Action also stated that all cultures set forth in the present application employed 500 IU/ml GM-CSF. As a result, the Official Action alleged that it was essential that GM-CSF be added to the culture. While applicants note that the specification does not disclose GM-CSF as essential, in the interest of advancing prosecution, the claimed invention has been amended to recite that GM-CSF is present in the culture. As a result, applicants believe that this objection has been obviated by the present amendment.

Applicants acknowledge that believe that the present disclosure states that any concentration of type I IFN greater than 100 IU/ml can be used in the present invention. A range of 400 IU/ml-10,000 IU/ml is taught in the present specification at page 6, lines 5-15. As a result, applicants believe that this claim amount of greater than 400 IU/ml is enabled by the present disclosure.

The Official Action has taken a position that it was applicant's decision to disclose the use of just a single concentration of IFN and a single concentration of GM-CSF. Moreover, the Official Action cites to MPEP Statute 2164.03 as stating that physiological processes are generally unpredictable.

However, as noted above, applicants provide examples for more than one concentration of IFN and the claims now recite that the cells are cultured in the presence of GM-CSF.

Moreover, as a matter of law, the Examiner is reminded that the examiner has the *initial* burden to establish a reasonable basis to question the enablement provided for the claimed invention. *In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993) (examiner must provide a reasonable explanation as to why the scope of protection provided by a claim is not adequately enabled by the disclosure) (See MPEP § 2164).

While the Official Action notes that the specification provides examples that utilize a limited number of concentration amounts, applicants respectfully submit that the Office Action does not provide any evidence that would suggest that the present disclosure is not enabling for the claimed amounts and ranges. Indeed, as long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. 112 is satisfied. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). Failure to disclose other methods by which the claimed invention may be made does not render a claim invalid under 35 U.S.C. 112. *Spectra-Physics, Inc. v. Coherent, Inc.*, 827

F.2d 1524, 1533, 3 USPQ2d 1737, 1743 (Fed. Cir.), *cert. denied*, 484 U.S. 954 (1987) (See MPEP § 2164.01(b)).

As no evidence is presented as to why the disclosed method for making and using the claimed invention does not provide a reasonable correlation, applicants respectfully request that the rejection be withdrawn.

Claims 54-70 were rejected under 35 USC §102(b) as allegedly being anticipated by PAQUETTE et al. This rejection is respectfully traversed.

PAQUETTE et al. disclose the culture of peripheral blood mononuclear cells (PBMCs) with a combination of GM-CSF and IFN in order to obtain dendritic cells, although only a particular subtype of IFNα is used. Yet the method of the invention differs from that of PAQUETTE et al. in that the method of PAQUETTE et al. involves 7 days of culture whereas that of the invention involves 3 days of culture.

PAQUETTE et al. does not contemplate the possibility of culturing dendritic cells in as little time as 3 days. Nowhere in the PAQUETTE et al. reference is it disclosed or suggested that the cells be isolated within 3 days. Indeed, as the Examiner is aware, it was generally thought at the time the invention was made that full differentiation into dendritic cells takes 5 to 7 days, as is true of conventional cells obtained with

IL-4 and GM-CSF or of the cells of PAQUETTE et al. Thus, PAQUETTE et al. would lead one away from the claimed method.

Moreover, the product obtained by PAQUETTE et al. is distinct from that obtained owing to the method of the invention. For instance, CD14 is expressed less in the cells of the invention than in the cells of PAQUETTE et al. (36% in Table 1 of the present application vs. 56% in Table 1 of PAQUETTE et al.). As the Examiner is aware, a population of cells that expresses less of CD14 is generally considered to be more differentiated than a population of cells that expresses more of CD14. Therefore, it can be assumed from these data that the cells of PAQUETTE et al. (i.e., dedifferentiate) less differentiated than cells of the invention. As a result, applicants do not believe that it can be said that PAQUETTE et al. inherently discloses the same cell.

In regards to antigen presentation and the induction of an antigen-specific response, PAQUETTE et al. does not show the induction of a specific response to primary antigens. In fact, the immune response to tetanus toxoid (TT) is considered as a secondary response to recall antigens, as the great majority of individuals have been immunized against TT at least once in their life.

In contrast, the response to HIV-1 antigens which is evidenced in the present invention represents a response to

"primary antigens" (donors of the study were previously screened for HIV negativity). These experimental facts unambiguously show that the product obtained by PAQUETTE et al. is different from the product obtained according to the invention.

Thus, in view of the above, applicants believe that PAQUETTE et al. fail to anticipate or render obvious the claimed invention.

Claims 52-62, 64, and 68 were rejected under 35 USC §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This rejection is respectfully traversed.

Claims 54 and 69 have been amended as suggested by the Examiner. It is believed that these claims have been amended in a manner so as to obviate the claimed invention.

Claim 55 was rejected for reciting the term "any synthetic type I IFN". Applicants respectfully submit that a person skilled in the art would understand that this phrase includes any synthetic type I IFN produced from recombinant DNA, including in particular synthetic consensus IFN (CIFN) whose sequence is based on a consensus derived from amino acid sequences of human IFN-alpha. As evidence, the Examiner's attention is respectfully directed to the article: "The Biology of the Interferon System" (E. De Maeyer and H. Schellenkens, pp.

119-128. Elsevier, Amsterdam), which discusses the production of IFN by synthetic means. In particular, the Examiner's attention is directed to the second full paragraph at pg. 102.

Moreover, applicants believe that the specification does adequately address this term. Indeed, the term is explicitly recited along with other IFN types that may be utilized in the claimed method at page 6, lines 1-10 and pg. 17, lines 35-30. In view of the disclosure of IFN types that may be practiced with the claimed invention and the knowledge available to one skilled in the art, applicants believe that the expression is definite to a person skilled in the art.

The outstanding Official Action also rejected claim 62 for reciting the phrase "maturation agent". However, applicant submits that a person skilled in the art of *ex vivo* preparation of dendritic cells would know what kind of agent to employ and at what concentration levels to administer the maturation agent. Indeed, the specification discloses on page 4 that, after differentiation of dendritic cells from monocytes "further dendritic cell [DENDRITIC CELL] maturation can be driven by the addition of TNF-alpha, IL-1, LPS, monocyte-conditioned medium or sCD40L".

Thus, while the term may be broad, applicants believe that the term is definite to one skilled in the art. Indeed, breadth of a claim is not to be equated with indefiniteness. *In*

re Miller, 441 F.2d 689, 169 USPQ 597 (CCPA 1971). If the scope of the subject matter embraced by the claims is clear, and if applicants have not otherwise indicated that they intend the invention to be of a scope different from that defined in the claims, then the claims comply with 35 U.S.C. 112, second paragraph.

Claim 64 was allegedly vague and indefinite for reciting the term "growth factor". However, the term is described at page 5, lines 28-32. Thus, the term growth factor is definite to one of ordinary skill in the art.

In view of the above, applicants believe that the claimed invention is definite to one skilled in the art.

Claims 54-71 were rejected under 35 USC §112, first paragraph, for allegedly not satisfying the written description requirement. This rejection is respectfully traversed.

At this time, the Examiner is respectfully reminded that explicit support for an amendment to the claims or specification is not required. The description need not be in *ipsis verbis*. *Martin v. Johnson*, 454 F.2d 746, 751, 172 USPQ 391, 395 (CCPA 1972). Rather, the issue is not whether a specific new word or claim was used in the specification as filed but whether the concept as expressed by the word or

phrase was present *In re Anderson, 471 F.2d 1237, 176,USPQ 331 (CCPA 1973)*. Upon reviewing the specification, applicants believe that the concept of each of the phrases that are objected as allegedly introducing new matter into the present application is plainly supported by the present specification.

As noted above, present disclosure states that any concentration of type I IFN greater than 100 IU/ml can be used in the present invention. As the Examiner is aware, 400 IU/ml is greater than 100 IU/ml. Moreover, a range of 400 IU/ml-10,000 IU/ml is taught in the present specification at page 6, lines 5-15. As a result, applicants believe that the concept of greater than 400 IU/ml is supported by the present disclosure.

The present specification also discusses in numerous places the culturing of cells in the absence of IL-4 (e.g., pg. 19, lines 15-20; and pg. 20, lines 28-32). As a result, applicants believe that the concept of culturing cells in the absence of IL-4 is plainly presented and fully supported in the present disclosure.

The phrase "which promotes monocyte/dendritic cell survival" is explicitly supported on pg. 5, line 32.

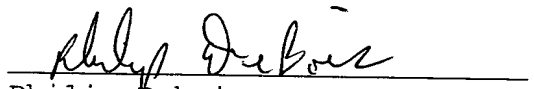
Thus, in view of the above, applicants believe that no new matter has been introduced into the present disclosure.

Thus, in view of the above, applicants believe that the present application is in condition for allowance at the time of the next Official Action. Allowance and passage to issue on that basis is respectfully requested.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

YOUNG & THOMPSON



Philip Dubois, Reg. No. 50,696
745 South 23rd Street
Arlington, VA 22202
Telephone (703) 521-2297
Telefax (703) 685-0573
(703) 979-4709

PD/mjr
May 20, 2005

APPENDIX:

The Appendix includes the following item:

- De Maeyer et al., "The Biology of the Interferon System 1983", Proceedings of the Second International TNO Meeting on the Biology of the Interferon System, held in Rotterdam, The Netherlands on 18-22 April 1983, pp. 119-128.

THE BIOLOGY OF THE INTERFERON SYSTEM 1983

Proceedings of the Second International TNO Meeting on the Biology of the Interferon System, held in Rotterdam, The Netherlands on 18-22 April 1983

Editors

Edward De Maeyer

and

Huub Schellekens

1983

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The Biology of the Interferon System 1983
E. De Maeyer and H. Schellekens, editors

PRODUCTION, CHARACTERIZATION AND BIOLOGICAL EFFECTS OF RECOMBINANT DNA DERIVED HUMAN IFN-α AND IFN-γ ANALOGS

K. ALTON, Y. STABINSKY, R. RICHARDS, B. FERGUSON, L. GOLDSTEIN, B. ALTROCK, L. MILLER AND N. STEBBING

Applied Molecular Genetics, Inc., 1900 Oak Terrace Lane, Thousand Oaks, Ca 91320 USA

INTRODUCTION

Molecular cloning of cDNA from human IFN-α mRNA and specific probing of the human genome has revealed a family of related IFN-α subtypes of 165 or 166 amino acid residues.[1] In contrast, only 1 human IFN-γ gene has been identified and this consists of 146 amino acid residues with little sequence relationship to the IFN-αs.[2] Although certain of the biological properties of interferons by definition are conserved, there are differences in the magnitude and nature of the properties of IFN-γ and the IFN-αs. Indeed, the various IFN-α subtypes show remarkable differences in view of their structural similarity.[3-5] In the case of IFN-αs, investigation of properties of different subtypes and molecular hybrids has demonstrated remarkable differences in pharmacological properties arising from differences, in some cases, of only three amino acid residues.[6] Such structure/activity studies have not been possible with human IFN-γ because it is not a family of related proteins. We have exploited rapid gene synthesis methods to construct genes coding for novel IFN-αs and IFN-γs and report here the production and properties of these interferon analogs.

THE DESIGN AND EXPRESSION OF IFN-α GENES

Overall 85/166 amino acid positions are conserved in the known IFN-α subtypes, listed in the first column of Table 1. There are at least 5 IFN-α pseudogenes, which cannot be translated into complete interferons because of deletions or stop codons.[7] Identification of IFN-α subtypes by different laboratories or different methods has revealed variants of individual IFN-α subtypes which differ in 1 to 4 amino acid positions.[1,5,8] Ignoring the pseudogenes there are 13 known distinct IFN-α subtypes and pairwise comparisons of these subtypes (Table 1) show differences in 4 to 38 positions. The 4 differences between IFN-αC_1 and IFN-αI and between IFN-αC and IFN-αL are dispersed whereas the 4 differences between variants, such as IFN-αB and IFN-αB_2 are in adjacent positions and in every case differences between variants arise from no more than two nucleotide differences between the

genes. In 7 positions (14,16,71,78,79,83,160) the various IFN-α subtypes show alternative amino acids, allowing classification of the subtypes into two subgroups in each of which the 7 positions are occupied by the same set of amino acid residues.[8] Three IFN-αs (IFN-αH, IFN-αF and IFN-αB) cannot be classified as group I or group II and in terms of the distinguishing positions they appear to be natural hybrids between the group I and group II subtypes. Overall IFN-αB shows the greatest differences on pairwise comparison with the other subtypes and may represent a third subgroup. The relationship between the subtypes in the same subgroup is also reflected by the smaller number of differences for pairwise comparisons between subtypes of the same subgroup compared with comparisons between subtypes of different subgroups(see Table 1).

Synthetic genes coding for IFN-αs were designed to optimize codon use for E. coli but also to allow construction of sub-gene duplexes with unique restriction sites at either end. Replacement of pairs of oligonucleotides in any subunit allowed construction of variant genes with differences in one or more amino acid residues. This allowed sequential cloning in pBR322 of successive gene segments, each approximately 1/3 of the entire coding region (see Fig. 1). Each subunit was obtained from a single "shot-gun" ligation

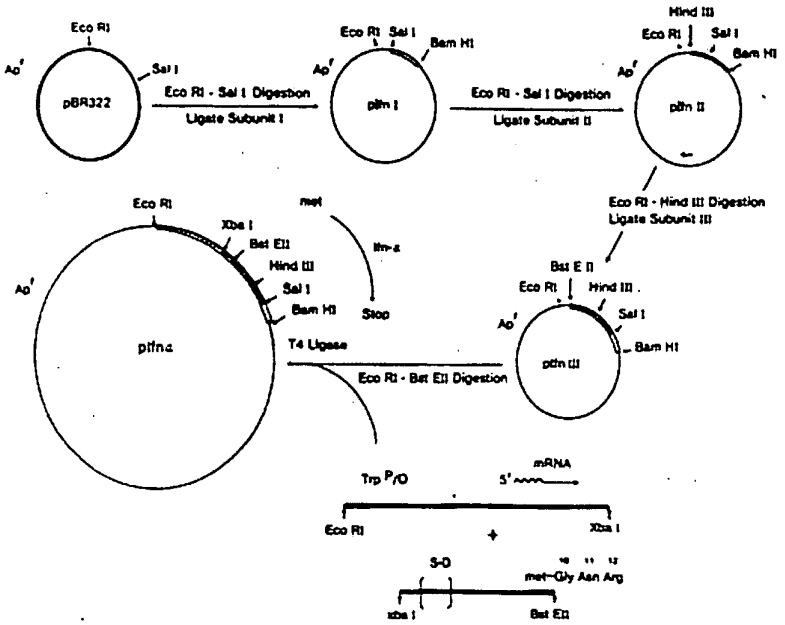


Fig. 1. Scheme for sequential cloning of synthetic sub-gene fragments.

TABLE 1

PAIRWISE COMPARISON OF THE NUMBER OF DIFFERENCES BETWEEN KNOWN HUMAN IFN-α SUBTYPES*

Group	Subtype	αA	αD	αS	α6	αC	αC_1	α4b	αI	αL	αJ	αH	αF	αB	Unique
	IFN-αA/(2)	--	29	26	23	31	33	31	32	35	34	28	30	31	7 (7)
I	IFN-αD/(1)		--	25	26	32	32	30	33	35	37	32	28	38	13 (14)
	IFN-αS/(G)			--	23	28	26	25	28	31	31	24	21	30	5 (4)
	IFN-α6/X				--	29	31	30	32	32	33	28	29	32	10
	IFN-αC					--	10	10	10	4	14	23	18	32	0
	IFN-αC_1						--	9	4	12	14	25	19	34	1
II	IFN-α4b/(4a)							--	9	14	11	25	16	32	1 (1)
	IFN-αI								--	14	15	26	19	34	0
	IFN-αL									--	16	24	22	35	2
	IFN-αJ/7										--	26	23	35	5
	IFN-αH/(2aH)/(H_1)											--	28	29	5 (5)
	IFN-αF												--	31	6
	IFN-αB/8/(B_2)													--	15 (12)
	$IFN\text{-}\alpha Con_1$	20	20	16	20	16	17	14	17	20	21	19	10	23	

*Numbers refer to amino acid differences
Designations of the same IFN-α from different labs are indicated in the vertical column of subtypes, e.g. IFN-α6 and IFN-αX.
Where variants of the same subtype have been identified, variants are shown in brackets.

reaction of synthetic single-stranded deoxyoligonucleotides designed to allow unique base-pairing by the sticky ends. Chemical synthesis of DNA was performed on a silica-gel support using 5'-dimethoxytrityl-3'-methoxy-N,N'-dimethylaminophosphoramidates as key intermediates.[9]

In considering the design of new IFN-αs we wished to incorporate features common to the known IFN-α subtypes. Although pairwise comparisons show an average of 25 differences between the known subtypes, the number of amino acid residues found uniquely at particular positions vary from 0 to 15 (see Table 1). IFN-αC and IFN-αI have no unique positions and thus represent different types of average IFN-α. However, IFN-αF overall shows the least differences from other subtypes when allowance is made for the relationship between subtypes in the same subgroup (see Table 1). We constructed a gene for IFN-αF and a novel consensus form of all the known subtypes which, in general, incorporates the most frequently observed amino acid residue at each position. This consensus IFN-α, designated $IFN\text{-}\alpha Con_1$, differs from IFN-αF in just 10 positions. That $IFN\text{-}\alpha Con_1$ is more of a consensus of the known IFN-α subtypes than IFN-αC or IFN-αI is demonstrated by the smaller number of differences (average = 18) between $IFN\text{-}\alpha Con_1$ and the known IFN-α subtypes, as indicated in the bottom line of Table 1, whereas even for IFN-αC and IFN-αI the average number of differences are 20 and 21, respectively. IFN-αF and $IFN\text{-}\alpha Con_1$ are hybrids in terms of the 7 positions that distinguish group I and II IFN-α subtypes but both are like group II IFN-αs in terms of positions 14 and

TABLE 2

AMINO ACID DIFFERENCES BETWEEN THE NATURAL SUBTYPE IFN-αF_1 AND VARIOUS ANALOGS

IFN Designation	Amino Acid differences														
	14	16	22	(71)	76	78	79	(83)	86	90	96	156	157	158	(160)
IFN-αF_1	A	I	G	(K)	T	E	Q	(E)	S	N	M	K	I	F	(E)
IFN-αF_2	T	M	G	(K)	T	E	Q	(E)	S	N	M	K	I	F	(E)
IFN-αCon_1	A	I	R	(K)	A	D	E	(E)	Y	Y	L	T	N	L	(E)
IFN-αCon_2	T	M	R	(K)	A	D	E	(E)	Y	Y	L	T	N	L	(E)
Group I	T	M		K		D	E	D							E
Group II	A	I		E		E	Q	E							K

The 7 positions distinguishing group I and group II IFN-αs are shown at the bottom of the Table. Where these residues are conserved between IFN-αF_1, -αF_2, -αCon_1 and -αCon_2 they are shown in brackets.

16. Therefore, we constructed variants of IFN-αF and IFN-αCon_1 in which both positions 14 and 16 were changed to conform with group I IFN-αs. The variant form of IFN-αF is designated IFN-αF_2 and the original form of IFN-αF is here referred to as IFN-αF_1. The variant of IFN-αCon_1 in which positions 14 and 16 are changed to conform with group I IFN-αs is termed IFN-αCon_2. The amino acids at the distinguishing positions in these 4 forms of IFN-α are shown in Table 2 together with the residues diagnostic for group I and group II IFN-αs.

The various IFN-α genes were expressed in *E. coli* under control of *trp*, *lac* uv5 or P_L promoters. High level expression was achieved by suitable choice of promoters in combination with particular translation initiation sequences. Expression levels of 20% of the soluble proteins of *E. coli* have thus been achieved for several of the IFN-αs.

THE DESIGN OF IFN-γ ANALOGS

A gene coding for human IFN-γ was synthesized using a synthetic strategy similar to that for IFN-αs. Three ligated gene segments, terminating in restriction sites, were cloned successively and variant genes constructed as before by substitution of particular oligonucleotides prior to their ligation. In this way genes for the known human IFN-γ (here designated IFN-γ_1) and several variants were constructed, as shown in Table 3. Because a basis for constructing analogs of IFN-γ did not exist, as for the IFN-αs, we examined residues which from a structural point of view might be important and residues known to be important for activity in IFN-αs. Preliminary studies indicated

TABLE 3

AMINO ACID SUBSTITUTIONS IN IFN-γ ANALOGS AND THEIR RELATIVE ANTIVIRAL ACTIVITIES

IFN Designation	Amino acid position differences							Relative antiviral activity*
	1	2	3	28	39	48	81	
IFN-γ_1	C	Y	C	N	W	M	N	100
IFN-γ_4	-	-	-				K	49
IFN-γ_5					F		K	8
IFN-γ_6						T	K	6
IFN-γ_{10}							K	70

*Assayed in HeLa cells infected with EMC virus, per unit binding to the monoclonal antibody, as determined in an immunosorbent assay.

that an analog with lysine (K) in place of asparagine (N) at position 81 (IFN-γ_{10}) had increased activity against herpes virus. Further analogs were constructed which all incorporated this change at position 81. The only two cysteines in IFN-γ, at positions 1 and 3, are unlikely to be involved in a disulfide bridge but their possible involvement in intermolecular bridges could cause aggregation and affect activity. The replacement of the cysteines by other residues or the elimination of the first 3 residues (IFN-γ_4) allows investigation of the importance of the two cysteines. Most IFN-αs contain 2 tryptophans and in the case of IFN-αA chemical modification indicates that at least one of the tryptophans is important for activity.[10] In IFN-γ_5 the single tryptophan (W) in IFN-γ is changed to phenylalanine (F). Most of the IFN-αs contain 4 methionines. IFN-γ also contains 4 methionines although their relative location seems unrelated to those in the IFN-αs.[11] In IFN-γ_6 the methionine at position 48 has been changed to threonine.

PROPERTIES OF THE IFN-α AND IFN-γ ANALOGS

The antiviral activity of the 4 IFN-αs indicated in Table 2 was assessed in a range of mammalian cell cultures challenged with Vesicular Stomatitis or EMC virus and the results are shown in Table 4. The relative antiviral activity of IFN-αF_1 in these cell lines is essentially the same as observed in earlier studies.[3] In particular note the relatively low activity in MDBK (bovine) cells, which is pecular to IFN-αF, and the greater activity in Vero (monkey) cells against EMC virus compared with VS virus. IFN-αCon_1 and

TABLE 4

ANTIVIRAL ACTIVITY IN VARIOUS CELL LINES OF NATURAL AND CLONED IFN-αF AND ANALOGS

Virus	Cell Line	IFN-α			
		Buffy Coat	F_1	Con_1	Con_2
VSV	WISH	100	100	100	100
	HeLa	400	100	200	100
	MDBK	1,600	33	200	300
	Vero	10	0.1	10	0.1
EMC	WISH	100	100	100	100
	HeLa	100	5	33	33
	Vero	100	20	1,000	10

*Antiviral activity was determined by an end-point cytopathic effect assay as described previously.[3,24] Data for each virus normalized for antiviral activity on WISH cells.

IFN-αCon_2 do not show lower relative activity in MDBK cells compared with the human cells (WISH and HeLa). In Vero cells challenged with EMC or VS virus IFN-αCon_2 shows 100 fold lower antiviral activity compared with IFN-αCon_1, and lower antiviral activity for IFN-αCon_2 has been confirmed in other cells and against other viruses.[12]. A polyvalent antibody preparation, raised in sheep against natural buffy-coat IFN-α, (NIH G026-502-568) was found to immunoprecipitate antiviral activity of these interferons. The antiviral activity of the IFN-αs was found to be stable after treatment at pH2 or SDS, confirming that the analogs have diagnostic properties of IFN-αs.

A mouse monoclonal antibody (NK-2) raised against the lymphoblastoid form of IFN-α has been used in an immunoradiometric assay for IFN-αs.[13] The NK-2 antibody failed to recognize our 4 cloned IFN-αs, as indicated in Fig. 2. However, a rat monoclonal antibody[14], YOK, raised against buffy coat IFN-α interferon, was found to bind to the analogs, IFN-αCon_1 and IFN-αCon_2, as shown in Fig. 2. When interferons are assayed by activity the extent of binding by a monoclonal antibody will depend on its affinity for the interferon and the specific activity of the interferon. Other studies have shown that subtypes other than IFN-αD do not bind efficiently to the YOK antibody.[14] IFN-αCon_1 and IFN-αCon_2 show only a 10% difference in binding by the YOK antibody but the extent of binding is greater than for the natural standard IFN-α when comparable units of antiviral activity are considered. These data indicate that IFN-αCon_1 and IFN-αCon_2 could have higher intrinsic specific

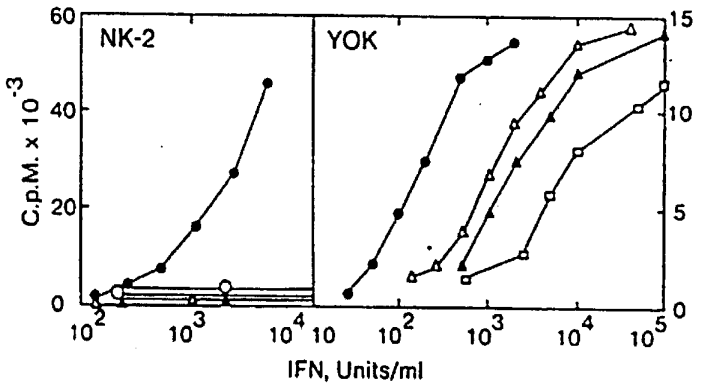


Fig. 2. Titration of standard IFN-α preparations and various cloned IFN-α analogs using two different IFN-α monoclonal antibodies, NK-2 and YOK. The extent of antibody binding (cpm) was determined for various dilutions of the interferons titrated in HeLa cells using EMC virus and the units corrected against the NIH IFN-α standard (G023-901-527).
●, IFN-αF_1; Δ, IFN-αCon_1; ▲, IFN-αCon_2;
O, standard natural IFN-α preparation

activities than the component subtypes present in the natural IFN-α standard preparation.

The high inherent specific activity of IFN-αCon_1, implied by the YOK antibody titration, has been confirmed directly by purification of the interferon to homogeneity as assessed on PAGE. By silver staining of gels the purified material showed only one band at a position corresponding to a MW of 19,600. The amount of this material was quantitated by analysis of the amino acid composition. Using the purified IFN-αCon_1, the specific activity, titrated in WISH cells challenged with EMC or VS virus, and corrected against the NIH standard (G023-901-527), is at least 3×10^9 U/mg protein. This compares with specific activities of no more than 2×10^8 U/mg protein for known forms of IFN-α titrated on human cells.[3,4,10,14]

Various biological activities of IFN-αCon_1 have been investigated and compared with other IFN-α preparations to determine which activities are linked to antiviral activity and which to the amount by weight of the interferon, i.e. structural domains independent of those conferring antiviral activity. Stimulation of natural killer (NK) cell activity by IFN-αCon_1 against K562 as target cells, was found to be comparable per unit of antiviral activity to natural buffy coat derived IFN-α. Efficacy of IFN-αCon_1 has also

been assessed in EMC virus infected squirrel monkeys and vaccinia virus infected Rhesus monkeys. The antiproliferative activity of the IFN-αs was compared with their antiviral activity because of the known inverse correlation observed for these activities using natural IFN-α subtypes.[16] Comparison of antiviral and antiproliferative activity of the various IFN-α and IFN-γ subtypes has demonstrated no correlation between antiviral and antiproliferative activity.[12] Moreover, the correlation of these activities with classification of the IFN-αs into groups does not seem to apply to the new analogs reported here.[12]

In order to compare the antiviral activity of the various IFN-γ analogs and allow for differential expression in E. coli binding to a monoclonal antibody was used to quantitate the amounts of the various analogs. The monoclonal antibody used was raised against a synthetic peptide from the carboxyterminal region of IFN-γ.[17] This peptide is common to all the analogs investigated. Western blot analyses[18] were used in conjunction with an immunosorbent assay to confirm and quantitate specific binding. In this manner the relative antiviral activities shown in Table 3 were derived. Clearly, changing the tryptophan at position 39 or the mentionine at position 48 decreased antiviral activity over 10 fold whereas elimination of the first 3 residues and hence the 2 cysteines only slightly decreased antiviral activity.

DISCUSSION

Although IFN-α subtypes of group I (see Table 1) have been shown to have greater antiviral than antiproliferative activity,[16] IFN-αCon_1 shows the highest known antiviral activity but the residues at positions 14 and 16 are those of group II IFN-αs. On changing the residues at positions 14 and 16 to those found in group I interferons (IFN-αCon_2) the antiviral activity in several cell systems is decreased, indicating that the inverse relationship between antiviral and antiproliferative activity of group I and group II IFN-αs does not arise solely from the residues at the 7 positions that distinguish the 2 subgroups of IFN-α.

Natural IFN-α preparations contain mixtures of the different subtypes identified by gene cloning.[19,20] Amino acid sequence data show that lymphoblastoid IFN-α contains little or no group I subtypes and IFN-αB, -αF or -αI must be the predominant subtypes.[5,21] It is known that the mouse monoclonal antibody, NK-2, binds 75% of the IFN-α activity in lymphoblastoid IFN-α[22] and to a large extent neutralizes the activity of this interferon

preparation.[13] But an NK-2 affinity column does not retain subtypes with activity on mouse cells[22] which could include IFN-αD and IFN-αF.[3] These observations indicated that IFN-αF is unlikely to be a significant component of lymphoblastoid interferon. The rat monoclonal antibody, YOK, does bind to IFN-αCon_1 and -αCon_2, although NK-2 shows little or no affinity for these subtypes. Although the full range of IFN-α subtypes bound by NK-2 and YOK and the differences in their specificities is still not completely determined, it is apparent that the two monoclonal antibodies do not distinguish group I and group II IFN-α subtypes.

Analogs or hybrids of known human IFN-αs were previously generated by splicing together homologous regions from genes for related IFN-α subtypes utilizing common restriction enzyme sites in the relevant genes.[15,23] However, the complete chemical synthesis of interferon genes allows synthesis of novel materials without limitations of restriction site locations in the genes or availability of gene segments coding for novel IFN-α domains. Changes of even a few amino acid residues can result in dramatic changes in activity. The single tryptophan residue in IFN-γ and the methionine at position 48 appear to play important roles in the antiviral activity of IFN-γ whereas presence of the cysteines at the N-terminus is relatively unimportant. Changing two residues in IFN-αs, at positions 14 and 16, can have major effects on activity (Table 4). Clearly chemical synthesis of genes provides a powerful method for exploring structure/activity relationships for biologically active polypeptides. Complete gene synthesis has already resulted in production of a novel consensus form of human IFN-α (IFN-αCon_1) with a specific activity about 20 times greater than known natural IFN-α subtypes.

REFERENCES

1. Goeddel, D.V., Leung, D.W, Dull, T.J., Gross, M., Lawn, R.M., McCandliss, R., Seeburg, P.H., Ullrich, A., Yelverton, E. and Gray P.W. (1981) Nature 290, 20-26.

2. Gray, P.W., Leung, D.W., Pennica, D., Yelverton, E., Najarian, R., Simonsen, S.S., Derynck, R., Sherwood, P.J., Wallace, D.N., Berger, S.L., Levinson, A.D. and Goeddel, D.V. (1982) Nature 295, 503-508.

3. Weck, P.K., Apperson, S., May, L. and Stebbing, N. (1981) J. Gen. Virol. 57,233-237.

4. Lee, S.H., Kelley, S., Chiu, H. and Stebbing, N. (1982). Cancer Res. 41, 1312-1316.

5. Stebbing, N. and Weck, P.K. (1983) In: Recombinant DNA Products, Insulin, Interferons and Growth Hormones, A. Bollon, ed., CRC Press.

6. Lee, S.H., Weck, P.K., Moore, J., Chen, S. and Stebbing, N. (1982) UCLA Symp. Mol. Cell Biol. 25, 341-351.

7. Brack, C., Nagata, S., Mantei, N. and Weissmann, C. (1981) Gene 15, 379-394.

8. Weissmann, C., Nagata, S., Boll, W., Fountoulakis, M., Fujisawa, A., Fujisawa, J.I., Haynes, J., Henco, K., Mantei, N., Ragg, H., Shein, C., Schmid, J., Shaw, G., Streuli, M., Taira, H., Todokoro, K. and Weidle, U. (1982) UCLA Symp. Mol. Cell Biol. 25, 295-326.

9. Matucci, M.D. and Caruthers, M.H. (1982) J. Am. Chem. Soc. 103, 3185-3191.

10. Wetzel, R., Levine, H.L., Estell, D., Shire, S., Finer-Moore, J., Stroud, R.M. and Bewley, T.A. (1982) UCLA Symp. Mol. Cell Biol. 25, 365-376

11. DeGrado, W.F., Wasserman, Z.R. and Chowdhry, V. (1982) Nature 300, 379-81.

12. Fish, E.N., Goldstein, L., Alton, K., Banerjee, K. and Stebbing N. (1983) These proceedings.

13. Secher, D.S. (1981) Nature 290, 501-503.

14. Hawkins, R.E., Spragg, J. and Secher, D.S. (1983) These proceedings.

15. Weck, P.K., Apperson, S., Stebbing, N., Gray,P.W., Leung, D., Shepard, H.M. and Goeddel, D.V. (1981) Nucleic Acids Res. 9, 6153-6166.

16. Fish, E.N., Banerjee, K. and Stebbing, N. (1983) Biochem. Biophys. Res. Commun. (in press).

17. Altrock, B., Hockman, H., and Stebbing, N. (1983) These proceedings.

18. Burnette, W.N. (1981) Anal. Biochem. 112, 195-203.

19. Allen, G., and Fantes, K.H. (1980) Nature 287, 408-411.

20. Rubinstein, M., Levy, W.P., Moschera, J.A., Lai, C.Y., Hershberg, R.D., Bartlett, R.T. and Pestka. S. (1981). Arch. Biochem. Biophys. 210, 307-318.

21. Zoon, K.C., Smith, M.E., Bridgen, P.J., Anfinsen, C.B., Hunkapillar, M.W. and Hood, L.E. (1980) Science 207, 527-528.

22. Allen, G., Fantes, K.H., Burke, D.C. and Morser, J. (1982) J. Gen. Virol. 63, 207-212.

23. Franke, A.E., Sherpard, H.M., Houck, C.M., Leung, D.W., Goeddel, D.V. and Lawn, R.M. (1982) DNA 1, 223-230.

24. Campbell, J.B., Grunberger, T., Kochman, M.A. and White, S.L. (1975) Can. J. Microbiol. 21, 1247-1253.

Complementary Experiment

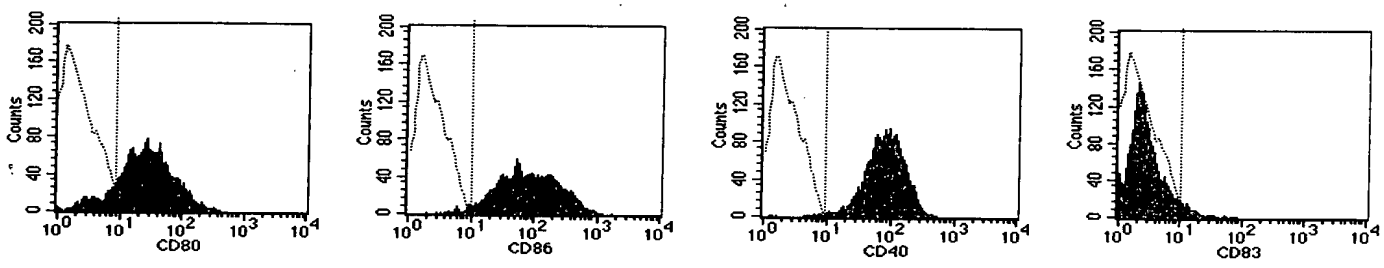
IFN-DCs generation

Peripheral blood mononuclear cells were obtained from heparinized blood of healthy donors by Ficoll density gradient centrifugation (Seromed). Monocytes were isolated by immunomagnetic selection (MACS Cell Isolation Kits; Miltenyi Biotec). Positively selected CD14+ monocytes (>98% as assessed by flow cytometry) were plated at the concentration of 2 x 10^6 cells/ml in AIM-V medium (GIBCO BRL), supplemented with 2% autologous plasma, 1000 U/ml GM-CSF, and 10,000 U/ml natural IFN-α (Alfaferone; AlfaWasserman) for 3 days.

Immunophenotypic Analysis.

Cells were washed and resuspended in PBS containing 1% human serum and incubated with a series of fluorochrome-conjugated mAbs to human antigens for 30 min at 4°C. The following mAbs were used for immunofluorescent staining: anti- CD80 (Becton Dickinson), CD40, CD86 (BD PharMingen), and CD83 (Immunotech). Cells were analyzed by flow cytometry. Data were collected and analyzed by using a FACSortTM (Becton Dickinson) flow cytometer, and analysis was performed by CELLQuestTM software (Becton Dickinson). Cells were electronically gated according to light scatter properties in order to exclude cell debris and contaminating lymphocytes. Fig.1 shows the expression of very high levels of co-stimulatory membrane molecules and confirmis previous data demonstrating the generation of dendritic cells by 3-day exposure of peripheral blood monocytes to 10,000 U/ml IFN-α and 1000 U/ml GM-CSF.

Fig. 1



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